## REMARKS

Claims 1-30 are pending in this application. Claim 1 has been amended and claims 2 and 17-20 have been canceled herein. The Office Action of March 24, 2003 has been reviewed and the Examiner's comments carefully considered. The present invention is a promoter of α-amylase derived from *Bacillus amyloliquefaciens* wherein the promoter is modified by the insertion of at least one restriction site located about 10 bases from the 3' end of the promoter, in which the modified promoter exhibits higher α-amylase translation activity than a promoter which does not have the restriction site. The present Amendment cancels claims 2, and 17-20, and amends claim 1. Support for the language in claim 1 is found on page 6, lines 18-21, page 7, lines 15 to 21, and Sequence ID Nos. 1 and 2 of the specification. No new matter has been added. In view of these amendments and the following explanation, Applicants believe that all the remaining claims are in condition for allowance.

Claims 1, 2 and 3-30 stand rejected under 35 U.S.C. 112, second paragraph, as assertedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. The Examiner asserts that the metes and bounds of the phrase "promoter of α-amylase derived from" are not clear. Claim 1 as amended now recites that the modified promoter of the present invention is derived from *Bacillus amyloliquefaciens*. Thus, the term specifically refers to one microorganism and does not encompass promoter sequences from recombinants, variants and mutants of any α-amylase DNA promoter. The Examiner, therefore, is correct, based on the context in which the term is used, to interpret "as derived from" as meaning "to isolate from or obtain from a source."

The Examiner asserts that the phrase "restriction site is introduced between a vicinity" renders the claim confusing and is not clear, and that the phrase "an activity of the promoter" is not clear. Claim 1 has been amended to incorporate the changes suggested by the Examiner, i.e., the phrase "restriction site is introduced between a vicinity" has been changed in order to clarify that the claim refers to a product; and "activity of the promoters" has been changed to "wherein the translation activity of the modified promoter of α-amylase is higher than...". The Examiner asserts that the metes and bounds of the phrase "higher than" is not clear. Applicants submit that this phrase does not render claim 1 indefinite

because, under the circumstances, claim 1 is intended to embrace any resultant α-amylase translation expression level which is elevated at all relative to the unmodified α-amylase promoter, and thus Applicants believe that they are entitled to all elevated values. The Examiner asserts that it is not clear as to what promoters are considered as "natural promoters." Claim 1 has been amended to substitute the term "modified promoter" for the term "natural promoter" in order to eliminate any confusion concerning the origin of the promoter and the *Bacillus* from which it is derived, i.e., that they are natural and not synthetic.

Claims 1, 3, 5-6, 8-16, 21-24, and 26-30 stand rejected under 35 U.S.C. 112, first paragraph, for asserted lack of enablement. Claim 1 as newly amended specifically recites that the modified promoter is derived from the microorganism *Bacillus amyloliquefaciens*, in which the "at least one restriction site is located about 10 bases from the 3' end" of the promoter DNA sequence. Claim 1 as newly amended, therefore, now refers to a single specific α-amylase promoter and not to recombinants, variants and mutants thereof. With regard to tolerance of the bacterium to modification, Applicants refer the Examiner to Table 1 in the specification, which provides comparison examples between the modified promoter of the claimed invention and a wild-type α-amylase promoter. The data show that the modified promoters of the examples have enzymatic activities well over four times that of the unmodified promoters, thus demonstrating the tolerance of *Bacillus amyloliquefaciens* to modification. Applicants therefore submit that claim 1 as now amended provides more than sufficient guidance to one skilled in the art to practice the claimed invention, and, furthermore, once one appreciates the benefit of adding a restriction site near the 3' end of the α-amylase promoter, one skilled in the art would have little difficulty adapting the technique to α-amylase promoters having different sequences.

Claims 1-3, 5-6, 8-16, 21-24, and 26-30 stand rejected under 35 U.S.C. 103(a) as assertedly being unpatentable over Palva et al. In contrast to the claimed invention, which inheres in a promoter of α-amylase derived from *Bacillus amyloliquefaciens*, wherein the promoter is modified by the insertion of at least one restriction site located about 10 bases from the 3' end of the promoter, resulting in a modified promoter that exhibits higher α-amylase translation activity than the same promoter not having the insertion of at least one restriction site, Palva et al. neither teach nor suggest adding of at least one restriction site near

the 3' end of an α-amylase promoter. It has been over eighteen years since the publication of the full promoter sequence of α-amylase derived from *Bacillus amyloliquefaciens*. Moran et al., Molecule. Gene. Genet. (1982) 186: 339. If it was obvious and efficacious to introduce a restriction site in the 3' end region of the a-amylase promoter in order to increase the translation activity therein, one skilled in the art would have attempted and/or succeeded long before now. In fact, Palva et al. actually teach away from making such a modification for the reasons that follow. It is well known that the nucleotide sequence and length (number of bases) of the sequence is very important for promoter expression, and it is also known that the *Bacillus subtilis* microorganism (disclosed in the Palva et al. reference and related to *Bacillus amyloliquifaciens*) has more stringent requirements than, for example, *E. coli* for recognition of transcription and translation initiation signals. Moran et al., Molecule. Gene. Genet. (1982) 186: 339. (The *B. subtilis* RNA polymerase demands high fidelity to the canonical-35 and –10 promoter hexanucleotides, and perhaps other sequences as well, and the *B. subtilis* ribosome requires extensive complementarity to messenger RNA). Because of the more stringent requirement of *B. subtilis* for recognition of translation initiation signals, one of ordinary skill in the art would never be motivated to modify the sequence responsible for the translation initiation signals, such as the polymerase binding site or ribosome binding site, nor would they consider modification of other sequences, because such modifications change the three-dimensional conformation of the promoter, thereby inhibiting or preventing the binding of RNA polymerase and ribosomes and adversely affecting the translation activity of the promoters. In particular, Palva et al. disclose the full sequence of the promoter derived from *Bacillus amyloliquefaciens* in Fig. 2, third line from the top: 5'-G AGA GGG AGA GGA AAC-3'. The sequence G AGA GGG AGA GGA from the 5' end, indicated by the solid and broken line in Fig. 2, constitutes a Shine-Dalgarno sequence, thought to be a potential RNA polymerase recognition site. Scherer et al., Nucl. Acids Res. (1980) 8: 3895-3907. One of ordinary skill in the art would not be motivated to modify this sequence by adding one or more restriction sites because such a modification of the RNA polymerase recognition site has been shown to decrease the promoter activity, as taught by Moran et al. The sequence 5'-G AGA GGG AGA GGA AAC-3' disclosed by Palva et al. contains the 10 nucleotides from the 3' end (underlined nucleotides) that are modified in the present invention. Thus, those skilled in the art would be motivated to preserve, rather than to

modify, the original sequence of α-amylase DNA promoters so as to ensure optimal promoter activity, based on the above-referenced prior data showing that modification of the nucleotide sequence and length may inhibit or prevent the binding of RNA polymerase and ribosomes, thus decreasing promoter activity.

For all the foregoing reasons, amended claim 1 and, therefore, claims 3-16 and 21-30, which depend either directly or indirectly from claim 1, are patentable over the cited prior art and in condition for allowance. Withdrawal of the asserted rejections and allowance of all pending claims 1, 3-16 and 21-30 are respectfully requested.

Finally, it is respectfully requested that the Examiner initial and return copies of the two Forms PTO/SB/08 which accompanied Applicants' Information Disclosure Statements filed February 11 and February 26, 2002.

Respectfully submitted,

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